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PHOTOSYNTHETIC RESPONSE OF SOYBEANS WITH GENETICALLY ALTERED CHLOROPHYLL[1]

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Abstract. The photosynthetic response of soybean plants (*Glycine max* (L.) Merr. Strain T219) at the start of a photoperiod showed that both normally pigmented plants (dark green) and chlorophyll-deficient plants (light green) had an induction period averaging 6 min, before a net CO_2 uptake could be measured. The induction period was followed by a rapid increase in photosynthesis until a maximum rate was reached after 15 min and then gradually declined. Both chlorophyll content and ribulose diphosphate carboxylase activity were greatest in dark green plants. Plants tested at mid-day exhibited the highest carboxylase activity and plants assayed at mid-night the lowest activity. During the first 5 min of illumination (following a 12 hr dark period) the carboxylase activity increased 1.5 times in dark green and 1.9 times in light green plants. This indicated that initiation of the photoperiod enhanced ribulose diphosphate carboxylase activity.

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Green plants have a delayed start in photosynthesis on photoperiod initiation. This delay has been termed the induction phenomenon and often involves a lapse of several minutes before the plant shows a net CO_2 uptake and oxygen evolution (Rabinowitch 1956). The induction phenomenon exhibited by most higher plants has been discussed at considerable length by Rabinowitch (1956) and Walker (1973). Earlier, Osterhoust and Haas (1918) suggested that the photosynthetic process at photoperiod initiation may be limited by the absence of sufficient quantities of intermediates or the need for certain catalysts (enzymes) to be activated by light. In our study the photosynthetic response of soybeans to photoperiod initiation was examined in terms of CO_2 assimilation and ribulose diphosphate (RuDP) carboxylase activity.

MATERIALS AND METHODS

Soybean plants (*Glycine max* (L.) Merr. Strain T219) displaying incomplete dominance for leaf pigmentation were used in this study. The F_3 generation of a cross from Richland and Linman 533 varieties segregated 1:2:1 for chlorophyll content where the heterozygous plants exhibited a light green leaf color (LG), while one-half of the homozygous plants were normally pigmented dark green (DG) and one-half were virtually achlorophyllous, or lethal yellow (LY).

Plant Culture Conditions. Soybean seeds (T219 strain) were germinated in moist vermiculite and kept in constant environment chambers with a 12 hr photoperiod from 0900 to 2100 hr. Day temperature was 25°C, night temperature 20°C and luminance of 2400 ft-c was supplied by a combination of incandescent and fluorescent lights. LG and DG plants were transplanted into pots (containing horticultural perlite) 10 to 14 days after cotyledon emergence (post emergence) and watered twice daily with an excess of mineral nutrient solution (Noble 1969). Lethal yellow plants were not used since these plants do not mature under normal physiological conditions.

Photosynthetic Measurements. Photosynthetic measurements were made on an attached leaf at the third or fourth node 30–40 days post-emergence with the leaf under an illuminance of 2400 ft-c. For each experiment, a fully expanded leaf was sealed in a clear plexiglass cuvette which was part of a closed CO_2 monitoring system (3 liters in volume) as described previously by Noble and co-workers (1973). Air was circulated over the leaf at 1ℓ/min, and temperature was maintained at 25°C±2.

CO_2 concentration was continuously monitored by means of an infrared gas analyzer (Beckman, Model 215A) and held at 300±10 ppm. Assimilated carbon dioxode was replaced in the closed system by means of an automatic syringe drive mechanism. The CO_2 required to replace CO_2 photosynthetically

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assimilated was used as a measure of net photosynthesis (mg CO_2 dm^{-2} hr^{-1}). Each attached leaf of a test plant was sealed in the cuvette near the end of a light period and held in total darkness until measurements were begun upon initiation of the photoperiod the following morning. CO_2 uptake was recorded every 2 min for the first 10 min and every 5 min thereafter for 35–40 min.

Enzyme Assay. Fresh leaf material, from the fourth node leaf, was collected 6 hr into the photoperiod (mid-day), 6 hr into the dark period (mid-night), 5 min prior to photoperiod initiation (pre-illumination) and within 5 min post-illumination (induction period) and assayed for ribulose diphosphate (RuDP) carboxylase activity by the method of Bowes and co-workers (1972). The assay was initiated by adding an aliquot of leaf extract to vials which contained 1 ml of a reaction mixture containing $NaH^{14}CO_3$ (2 μ Ci/ml) at 30°C. After 3 min the reaction was stopped with 0.1 ml 6 M acetic acid, the sample volume evaporated to 0.5 ml at 90°C, and relative enzyme activity determined by monitoring the incorporation of ^{14}C into organic form, via liquid scintillation spectrophotometry.

Chlorophyll Determination. Chlorophyll extracts from leaves at the fourth node of both DG and LG plants were prepared according to a method modified from Witham and co-investigators (1971). Chlorophyll content was then determined spectrophotometrically with a Beckman DK–2A Ratio Recording Spectrophotometer. Chlorophyll *a*:*b* ratios were calculated using Arnon's equation (1949) as modified by Bruinsma (1961).

RESULTS AND DISCUSSION

Total chlorphyll content was approximately 6 times greater in leaves of DG plants than in LG. The ratio of chlorphyll *a* present in DG plants to that in LG plants was about 7:1, while that for chlorophyll *b* was 4.5:1 (table 1). The chlorophyll *a*:*b* ratio in DG plants was approximately 2:1, while in LG leaves it was 1:1.

The photosynthetic rate for DG plants over the entire 36 min experimental period was 45% higher than for LG plants. Photosynthetic measurements made just after the photoperiod began, showed that immediately after illumination there was no net CO_2 uptake for 4–8 min. After the lag period, averaging 6 min, DG plants exhibited an initial CO_2 uptake, which maximized at 15 min post-induction (21 min into the experiment) at a value 15% above the post-induction period photosynthetic rate. Once the maximum rate was reached, there was a gradual decline in photosynthesis with the photosynthetic rate for the entire 30 min post-induction period averaging 19.8 ± 0.45 mg CO_2 $dm^{-}hr^{-1}$.

LG plant responses during the first 36 min following illumination were similar to those of DG plants (fig. 1). Following an induction period, averaging 6 min, there was a gradual increase in photosynthesis until the maximum (30% above the average post-induction rate for the period) was reached 10 min following the induction period (16 min into the experiment). During the next 20 min there was a decline in photosynthesis. The rate of CO_2 assimilation for the 30 min interval following the lag period averaged 13.6 ± 0.38 mg CO_2 $dm^{-2}hr^{-1}$.

RuDP carboxylase activity was determined for both DG and LG plants at 4 different times during the 24 hr cycle. Higher RuDP carboxylase activity was found in DG plants than in LG plants at all 4 sample times (table 2). In both types of plants, enzyme activity was highest at mid-day and lowest at mid-night. Also, RuDP carboxylase activity was greatly enhanced with the initiation of the photoperiod following a 12 hr dark period. Within groups the percent decline in RuDP carboxylase activity was much greater for LG plants. The mid-night activity was ½ the mid-day activity in LG plants, while in DG plants the

TABLE 1

Chlorophyll content of leaves of dark green and light green soybean plants.

	Total **	*a***	*b***	*a*:*b* ratio
Dark Green	2.08±.07*	1.33±.04	0.75±.03	1.77:1
Light Green	0.36±.03	0.20±.01	0.17±.01	1.18:1

*Data expressed as mean and standard error of mean (n=24).
**Values are expressed in mg chlorophyll (total, *a* or *b*) per gram fresh weight.

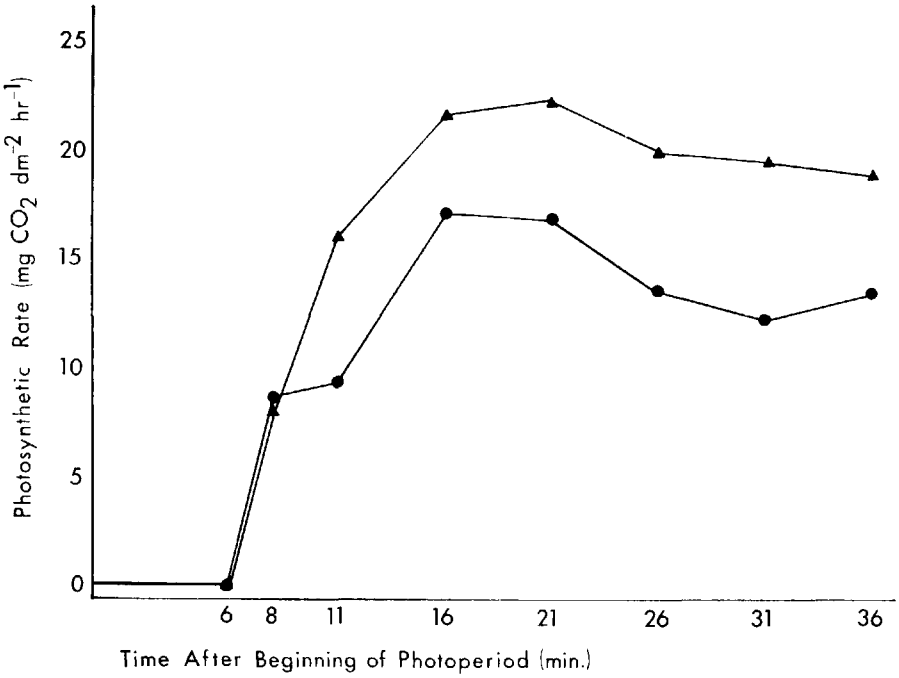

FIGURE 1. The photosynthetic response of soybeans to the initiation of the photoperiod (▲=DG soybeans, ●=LG soybeans).

mid-night activity was ⅓ the mid-day activity.

Soybean plants of the T219 strain responded to initial illumination in a manner similar to that described by Walker (1973), which he called a "simple induction phenomenon." This phenomenon is characterized by a period of several minutes, following photoperiod initiation, in which no net CO_2 assimilation occurs. The initial lag is followed by a sharp increase to an unusually high maximal rate, after which there is a more gradual decline until a nearly constant rate of CO_2 assimilation occurs.

Both DG and LG plants showed a marked increase in RuDP carboxylase activity when the leaf was illuminated following a 12 hr dark period (table 2). The data suggest light activation and dark deactivation of RuDP carboxylase; however, light activation of this enzyme appears to take place too rapidly to be accounted for by induction. Previous work with this enzyme (Pon 1959, Bassham and Jensen 1966, Sugiyama *et al* 1968) has shown that light activation is probably not a major factor in determining the presence, absence, or length of the induction period of plants (Walker 1973).

TABLE 2

RuDP carboxylase activity (dpm) of dark green and light green soybeans at 4 different times during the day.

	Mid-Day**	Mid-Night**	Before Illum.**	Post-Illum.†
Dark Green	288±15.4*	144±9.3	156±5.1	232±4.2
Light Green	204±11.1	72±5.7	78±5.6	148±3.0

*Data expressed as mean and standard error of mean.
**n=15.
†n=8.

The effects of concentration of the initial substrate and/or its precursors on induction has been discussed by Walker (1973), Selwyn (1966), and Rabinowitch (1956). A low level of RuDP at the onset of illumination would result in a low initial photosynthetic rate and, accordingly, a period of time would be needed for synthesis before a maximum photosynthetic rate could be achieved (McAlister 1937, Baldry *et al* 1966, Walker 1973). Upon initial illumination, both DG and LG plants show a characteristic lag (induction) phase of 6 min, during which time the autocatalytic sequence of events in the Calvin-Benson cycle would allow the production, and build-up, of intermediates needed for photosynthesis. The linear increase in photosynthesis (DG and LG) to the maximal rate at 10–15 min post-induction may be due to the increased initial carboxylation reaction as additional RuDP is produced (Walker 1973). As the available RuDP is rapidly utilized, a shortage of some other substrate may begin to develop, as indicated by the decline in photosynthetic rate, as observed in our study (fig. 1). The decline may also be a result of CO_2 becoming limiting as photosynthetic rates increase. During the induction phase excess CO_2 should accumulate in the photosynthetic tissues. As RuDP becomes available due to light activation, and photosynthesis increases, photosynthetic rates may become high temporarily, and then fall back to a lower, more stable, CO_2-rate limited condition.

Data collected in our study show DG plants to have 3 times the chrloophyll content reported by Keck *et al* (1970), for similar mutant soybeans, while the chlorophyll content of LG plants was 2 times previously reported values. It is believed that environmental conditions may account for these differences. It has been observed in a related study in our laboratory that chlorophyll content, especially in LG plants, increase with decreasing light intensity, and decreases with the age of the plant.

In our study the photosynthetic rates of DG and LG plants were slightly higher than those reported by Crang and Noble (1974) and the photosynthetic rate of DG plants was considerably higher than that of LG plants. Since conditions and method of measurement were the same in both studies, the difference is thought to be due to the time in the photoperiod during which photosynthetic measurements were made. The average photosynthetic rates reported in our study are based on CO_2 uptake during the first 36 min of the photoperiod at which time steady-state conditions probably have not yet been attained. Conversely, the photosynthetic rates reported by other investigators were based on measurements made on plants which were several hours into the period, plants which had achieved steady-state conditions.

Since leaves of LG plants are considerably thinner, it is likely that they have less intercellular air spaces. Therefore, if during the induction phase photosynthesis removes CO_2 from the air spaces, then less deficit would be created in the LG plants. Thus, less CO_2 would need to enter during post-induction making the photosynthetic rate appear to be lower. The higher photosynthetic rates of DG plants during the initial phase of illumination points to the existence of an apparently greater photosynthetic capacity usually not manifest later in the photoperiod. This may be accounted for in terms of differences in RuDP carboxylase activity in the 2 genotypes. The higher RuDP carboxylase activity in DG plants permits a more rapid assimilation of CO_2 once the stomates have opened.

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